A hotspot atop: rivers of the Guyana Highlands hold high diversity of endemic pencil catfish (Teleostei: Ostariophysi: Siluriformes)

MALORIE M. HAYES[1,*,], HOLDEN J. PAZ[1], CARLA C. STOUT[1,2], DAVID C. WERNEKE[1] and JONATHAN W. ARMBRUSTER[1]

[1]*Auburn University, Auburn, AL 36849, USA*
[2]*California State Polytechnic University, Pomona, Pomona, CA 91768, USA*

The Pakaraima Mountains are an ancient mountain range along the borders of Guyana, Brazil and Venezuela. The high plateau is drained by multiple river systems in all directions. Although hypotheses have been presented for the biogeographical relationships of lowland rivers, the interconnectivity of rivers on the top of the plateau is unknown. With multiple complex rivers in a small, upland area, we predicted a high level of endemism for stream fishes and complex biogeographical relationships. We explored this with the incredibly diverse pencil catfish genus *Trichomycterus*. Using collections from recent expeditions to the Pakaraima Mountains of Guyana, we amplified three mitochondrial (16S, *COI* and *Cytb*) and one nuclear marker (*rag2*). We constructed individual gene trees and a concatenated tree to determine the placement of these taxa within the *Trichomycterus* of the trans-Andean/ Amazonian clade. Herein, we identify six endemic lineages of *Trichomycterus* from the highlands of the Pakaraima Mountains. Of the identified lineages, we find two species occupying multiple basins, suggesting that Pakaraima streams either maintain connectivity or had some degree of recent connectivity.

ADDITIONAL KEYWORDS: biogeography – fishes – freshwater – Guiana Shield – highlands – Neotropics – systematics – *Trichomycterus* catfish.

INTRODUCTION

The Pakaraima Mountains run along the borders of Guyana, Brazil and Venezuela (Fig. 1). The streams there hold a high degree of endemism (Hardman *et al.*, 2002; Armbruster & Taphorn, 2011; Alofs *et al.*, 2014). The Pakaraimas are drained to the north by the Mazaruni and Cuyuní rivers, to the east by the Potaro River (Essequibo River basin), to the south-west by the Ireng and Uraricoera rivers (Amazon River basin) and to the west and north-west by the Caroní River (Orinoco River basin). The mountains are the remains of Archaean and Proterozoic rocks, whose lighter sediments have eroded to fill formerly lacustrine basins, such as the Venezuelan Llanos and the Rupununi Savanna of Guyana (for review, see Lujan & Armbruster, 2011). This erosion has left behind a durable core that often has steep faces, off which the rivers run in spectacular waterfalls. Below the falls, the rivers often have some rapids complexes, but quickly reach lowland conditions (Lujan & Armbruster, 2011).

Current evidence for the relationships of the rivers draining the Pakaraima Mountains involves, in part, the development and subsequent fragmentation of a palaeo-river drainage called the proto-Berbice (Sinha, 1968; Schaefer & do Vale, 1997; Lujan & Armbruster, 2011). The proto-Berbice contained what are now tributaries of the upper Rio Branco (Amazon basin, including the Ireng), the upper Essequibo, the Berbice and parts of the Courantyne and Orinoco. Meanwhile, the middle and lower Essequibo (including the Potaro/ Kuribrong) probably joined the Mazaruni and Cuyuní near where the mouths are at present. Slowly, the Amazon River has been capturing streams from the proto-Berbice in an east–west manner. This pattern would suggest a similarity between the faunas of the Potaro/Kuribrong and the upper Mazaruni, with the Ireng being more distantly related because it appears

*Corresponding author. E-mail: malorie.hayes@auburn.edu

862

Figure 1. A topographical map of the Pakaraima highlands, depicting the physiography of rivers. Major rivers are labelled along their flow. Country names are listed horizontally in capital letters.

never to have been connected into the middle and lower Essequibo plus Mazaruni.

However, the upper courses of the rivers have not been explored biogeographically. The relationships of the upper courses of the rivers, which are likely to be complex, were suggested by the description of the crenuchid *Apareiodon agmatos* and the loricariid taxa *Paulasquama callis*, *Neblinichthys brevibraccium* and *Neblinichthys echinasus* in the upper Mazaruni (Taphorn *et al.*, 2008, 2010; Armbruster & Taphorn, 2011), all of which share affinities with the Orinoco River basin. Given the absence of these taxa in lowland streams, it is likely that these highland taxa were moving via stream capture or other events that connected these highland tributaries. Thus far, the relationships of the highland regions have scarcely been explored in a systematic manner. Lujan *et al.* (2018) found that *Paralithoxus bovallii* (Loricariidae) from the Ireng was more closely related to an undescribed species

in the Courantyne than to one from the lower Potaro, supporting the proto-Berbice hypothesis; however, *Paralithoxus* is not found elsewhere in the Pakaraima highlands. Lujan *et al.* (2019) found that *Corymbophanes* (Loricariidae), an upper Potaro/ Kuribrong endemic, is sister to a new genus and species (*Yaluwak primus*) from the upper Ireng, with the two clades separated by long branch lengths, suggesting an ancient relationship.

Coupled with the lack of basic information on the fauna of the region, the area is also under extreme threat by gold and diamond mining, with a strong potential for mining to eliminate species before they are even discovered (Alofs *et al.*, 2014). In this study, we explore the potential interconnectedness of the high Pakaraima streams by examining the relationships of the pencil catfishes of the genus *Trichomycterus* in order to identify pertinent diversity and to uncover biogeographical patterns that could be duplicated in other Pakaraima organisms.

Trichomycteridae represents a diverse family of freshwater catfishes distributed across the Neotropics. Of the > 300 recognized species (Fricke *et al.*, 2018), the majority of species (219) are found in the Trichomycterinae, which contains the genera *Bullockia*, *Cambeva*, *Eremophilus*, *Hatcheria*, *Ituglanis*, *Rhizosomichthys*, *Scleronema*, *Silvinichthys* and *Trichomycterus*. Most of the diversity within Trichomycterinae can be attributed to *Trichomycterus*, with all other genera except *Ituglanis* (28 species), *Cambeva* (25 species), *Silvinichthys* (seven species) and *Scleronema* (three species) being monotypic (Fricke *et al.*, 2018). Although other genera exhibit apomorphic specializations, the lack of specializations unique to *Trichomycterus* has long made researchers suspect, and later confirm with molecular studies, the non-monophyly of the genus (Baskin, 1973; de Pinna, 2016; Ochoa *et al.*, 2017; Henschel *et al.*, 2018; Katz *et al.*, 2018).

The emerging phylogenetic pattern matches those of other fishes with a similar distribution, such as doradid catfishes, characins and armoured catfishes, where distinct clades are geographically linked to a trans-Andean/Amazonian distribution or to south Atlantic coastal drainages (Ribeiro, 2006; Ochoa *et al.*, 2017; Katz *et al.*, 2018). Katz *et al.* (2018) attempted to solve some of the taxonomic problems of the Trichomycterinae by restricting *Trichomycterus* to a clade that contained the type species (South Atlantic coastal drainages), describing *Cambeva* for a clade sister to *Scleronema*, a clade that is sister to *Trichomycterus s.s.*, and referring the Andean, Patagonian, Amazonian and Guiana Shield species to '*Trichomycterus*' in quotation marks. '*Trichomycterus*' is paraphyletic and part of a clade that includes *Bullockia*, *Eremophlius s.s.* and *Ituglanis*. These results were similar to those found previously by Ochoa *et al.* (2017). These patterns are not surprising, given the tectonic and geological history of the continent, which highlights the importance of the Guiana and Brazilian Shields as original uplands of South America, formation of the Andes and uplift of the Eastern Cordillera (to name a few factors) in shaping the biogeography of Neotropical fishes (Lundberg *et al.*, 1998; Ribeiro, 2006; Lujan & Armbruster, 2011). We will not be referring to *Trichomycterus* in quotation marks, and we refer our study species to genera as in the study by Ochoa *et al.*, (2017)

Trichomycterus are long, slender catfishes generally found only in swift waters. Such habitat, even in the mountains, is patchy, and we suspect that the fishes would be likely to be isolated to drainages. Recent collections from this region have identified all specimens as *Trichomycterus guianensis* (Eigenmann, 1912), but we noted significant differences in colour and morphology in samples that we have obtained. Preliminary external visual examinations indicate

the possibility for unrecognized diversity and perhaps misidentification of *T. guianensis* in the rivers of this region. The only other species recognized in the region is *Trichomycterus conradi* (Eigenmann, 1912), and we have found some specimens from the Ireng and Kuribrong rivers that correspond to this species.

Recent studies have illuminated the need to identify unrecognized diversity within *Trichomycterus* and have highlighted the important role that geology and topography play in contributing to that diversity (Unmack *et al.*, 2009; Ochoa *et al.*, 2017; Katz *et al.*, 2018). With our collections of seemingly multiple, diverse *Trichomycterus* species in the region, we: (1) confirm the discovery of multiple endemic *Trichomycterus* species in the region using a multigene phylogeny; (2) examine the diversity and endemism of *Trichomycterus* in the Pakaraima Mountain region with respect to the unique geological features that have probably influenced their genetic structure; and (3) provide clarification for the identification of *T. guianensis* and *T. conradi* based on morphology.

MATERIAL AND METHODS

TAXON SAMPLING, DNA EXTRACTION AND SEQUENCING

Collections ranged across multiple years, with research permits from Environmental Protection Agency of Guyana as follows, listed as year, reference number: 2011, 030510 BR 130; 2008, 300408 SP: 004; 2014, 040414 SP: 003; 2015, 123115 BR031; and 2016, 012016 SP: 003. Fish were either collected with 2 m × 3 m nylon-coated seines with 3 mm mesh, or we joined fishing expeditions of the Patamona, who used hiari, a root native to the area around the collection site and a natural source of rotenone. Collection sites were distributed across the Pakaraiama highlands (Fig. 2; Table 1). After capture, fish were euthanized in a solution of tricaine methanesulfonate (MS-222) until no sign of respiration was observed for 5 min. Tissue samples were taken from the right pectoral fin or right axial musculature and placed into 1.5 mL vials containing RNALater or ethanol for preservation. Once tissue samples were taken, voucher specimens were fixed in a 3.7% formaldehyde solution for 7 days, then rinsed in water for 3 days, and finally stored in 70% ethanol. Vouchers and tissue samples were deposited in the AUM Fish Collection. Additional materials not collected by the authors were requested from the Royal Ontario Museum (ROM; Table 1).

Whole genomic DNA was extracted from tissues using either Chelex or an E.Z.N.A. Tissue DNA Kit (Omega BioTek, Norcross, GA, USA). The four genes, 16S, *COI*, *Cytb* and *rag2*, were amplified through
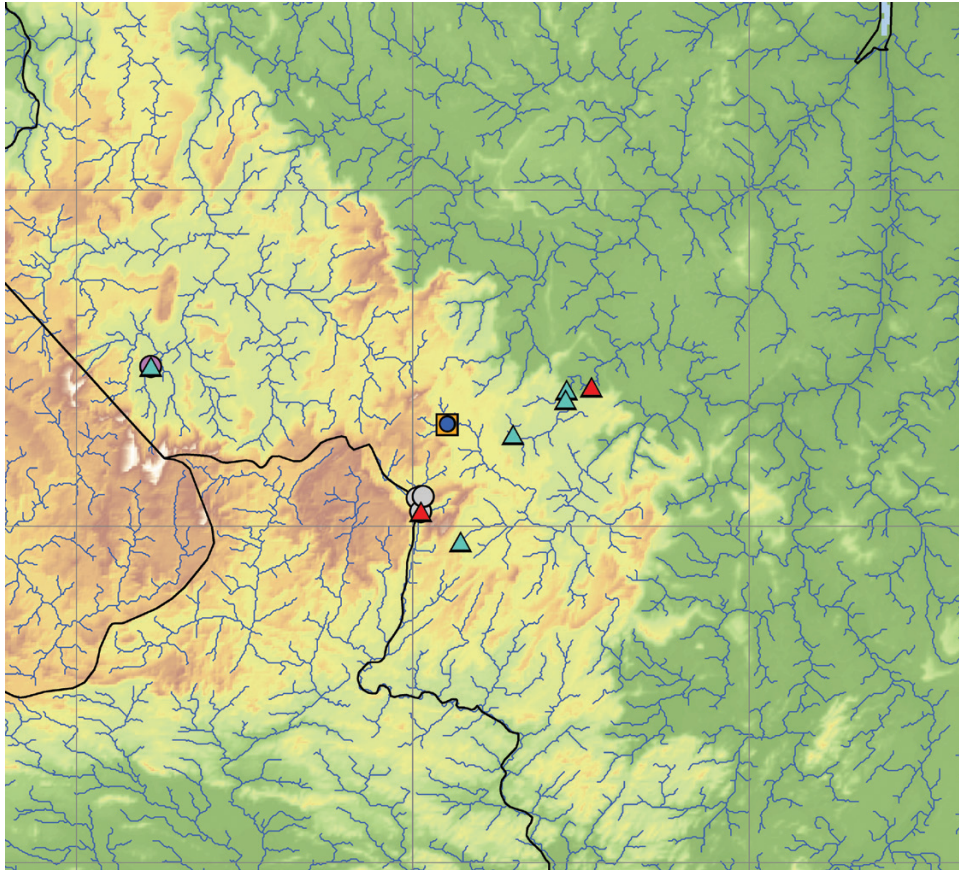
Figure 2. Collection localities for species of *Trichomycterus* found in this study. Colour codes correspond to images in Figure 4 and are as follows: red triangles, *Trichomycterus conradi*; blue circle, *Trichomycterus guianensis*; teal triangles, *Trichomycterus* cf. *guianensis*; purple circle, Mazaruni, plain form; orange square, Potaro, elongate; grey circles, Ireng, spotted form.

25 µL polymerase chain reactions (PCRs) using the same primers as Ochoa *et al.* (2017). The 16S gene was amplified using the following primers and protocol: 16Sa-L and 16Sb-H (Palumbi & Baker, 1994); initial denaturation step of 180 s at 94 °C, then 30 cycles of denaturation (45 s at 95 °C), annealing (30 s at 54 °C) and extension (60 s at 68 °C), followed by a final extension of 600 s at 68 °C. The *COI* gene was amplified using the following primers and protocol: FishF1 and FishR1 (Ward *et al.*, 2005); initial denaturation step of 180 s at 94 °C, then 30 cycles of denaturation (45 s at 94 °C), annealing (30 s at 54 °C) and extension (60 s at 68 °C), followed by a final extension of 60 s at 68 °C. The *Cytb* gene was amplified using the following primers and protocol: L14841 and H1591 (Kocher *et al.*, 1989; Irwin *et al.*, 1991); initial denaturation step of 180 s at 94 °C, then 30 cycles of denaturation (45 s at 95 °C), annealing (30 s at 54 °C) and extension (60 s at 68 °C), followed by a final extension of 60 s at 68 °C. The *rag2* gene was amplified using a two-step protocol. The first reaction was performed using the

touchdown protocol described by Lovejoy & Collette (2001), with RAG164F and RAG2R6 primers. The second PCR used 1.5 µL of template from the first run and the primers 176R and RAG2Ri (Oliveira *et al.*, 2011) in the following conditions: initial denaturation step of 30 s at 95 °C, then 35 cycles of denaturation (30 s at 95 °C), annealing (45 s at 56 °C) and followed by a final extension of 72 °C for 300s. Primers used for PCR amplification were also used for DNA sequencing for all genes, with 176R and RAG2Ri being used for sequencing *rag2*.

The products were visualized and size verified on a 0.8% agarose gel. The PCR purification, sample preparation and Sanger sequencing were performed at GeneWiz (South Plainfield, NJ, USA). Chromatographs from forward and reverse reads were imported into Geneious v.10.2.3 (Kearse *et al.*, 2012) for assembly. Assembled contiguous sequences were aligned using the MUSCLE algorithm (Edgar, 2004), and results were checked by eye. Owing to variation in length among sequences generated in this study and

Table 1. Collection information for *Trichomycterus* species used in this study, with GenBank accession numbers provided for each gene and individual

Tissue catalogue	Species identity	Voucher number	Latitude	Longitude	16S	COI	Cytb	rag2	Drainage	Locality name	Country
AUFT10166	Ireng, spotted	67129	5.08955	−59.97514	MT025525	MT017634	–	MT017607	Ireng River drainage	Sukwabi Creek, at the top of Andu Falls	Guyana
AUFT10168	Ireng, spotted	67129	5.08955	−59.97514	MT025526	MT017635	–	MT017608	Ireng River drainage	Sukwabi Creek, at the top of Andu Falls	Guyana
AUFT10169	Ireng, spotted	67129	5.08955	−59.97514	MT025527	MT017636	–	MT017609	Ireng River drainage	Sukwabi Creek, at the top of Andu Falls	Guyana
AUFT10170	Ireng, spotted	67129	5.08955	−59.97514	MT025528	MT017637	–	MT017610	Ireng River drainage	Sukwabi Creek, at the top of Andu Falls	Guyana
AUFT10212	*T. conradi*	67138	5.04398	−59.97717	MT025529	MT017638	–	MT017604	Ireng River drainage	Ireng River, shoals at the mouth of Monkey Creek, Kaibarupai	Guyana
AUFT10213	*T. conradi*	67138	5.04398	−59.97717	MT025530	MT017639	–	–	Ireng River drainage	Ireng River, shoals at the mouth of Monkey Creek, Kaibarupai	Guyana
AUFT10234	Ireng, spotted	67154	5.08388	−59.98762	MT025531	MT017640	–	MT017611	Ireng River drainage	Ireng River, above Uluk Tuwuk Falls	Guyana
AUFT10276	Ireng, spotted	67179	5.04398	−59.97717	MT025532	MT017641	–	MT017612	Ireng River drainage	Ireng River, shoals at the mouth of Monkey Creek, Kaibarupai	Guyana
AUFT10294	*T. conradi*	67194	5.08867	−59.96952	MT025533	MT017642	–	–	Ireng River drainage	Sukwabi Creek, East Fork, downstream of Wotowanda Falls	Guyana
AUFT10310	Ireng, spotted	67172	5.08955	−59.97514	MT025534	MT017643	–	MT017613	Ireng River drainage	Sukwabi Creek, at the top of Andu Falls	Guyana
AUFT2110	*T. guianensis*	63677	5.30181	−59.89838	MT025520	MT017630	MT017624	–	Potaro River drainage	Potaro River, at Ayanganna Old	Guyana
AUFT2186	*T. cf. guianensis*	62902	5.40532	−59.5439	MT025521	MT017631	MT017617	MT017603	Potaro River drainage	Grass Falls Creek [Kiwikparu Creek], near top of falls	Guyana

Table 1. Continued

Tissue catalogue	Species identity	Voucher number	Latitude	Longitude	16S	COI	Cytb	rag2	Drainage	Locality name	Country
AUFT4743	*T.* cf. *conradi*	51758	4.767118	–54.56462	MT025522	MT017632	–	–	Maroni River drainage	Samansoe Kreek at confluence of two streams, 7.25 km south-east from Suralco Base Camp, Nassau Mountain, 0.23 km south (downstream) from SUR10-27	Suriname
AUFT6563	*T. guianensis*	62932	5.30181	–59.89838	–	MT017633	MT017625	MT017606	Potaro River drainage	Streams surrounding Ayanganna Old	Guyana
AUFT6596	Potaro, elongate	62949	5.304	–59.89819	MT025523	–	MT017628	MT017615	Potaro River drainage	Moyow Creek, upstream of Ayanganna Old	Guyana
AUFT6597	Potaro, elongate	62949	5.304	–59.89819	MT025524	–	MT017629	MT017616	Potaro River drainage	Moyow Creek, upstream of Ayanganna Old	Guyana
ROMT06183	Mazaruni, plain	83791	5.4755	–60.77967	MT025535	MT017644	MT017626	MT017614	Mazaruni River drainage	Riffles and shallow rapids upstream from camp, Waruma Creek	Guyana
ROMT06184	Mazaruni, plain	83791	5.4755	–60.77967	MT025536	MT017645	MT017627	–	Mazaruni River drainage	Riffles and shallow rapids upstream from camp, Waruma Creek	Guyana
ROMT06185	*T.* cf. *guianensis*	83790	5.4755	–60.77967	MT025537	MT017646	MT017618	–	Mazaruni River drainage	Riffles and shallow rapids upstream from camp, Waruma Creek	Guyana
ROMT06186	*T.* cf. *guianensis*	83790	5.4755	–60.77967	MT025538	MT017647	MT017619	–	Mazaruni River drainage	Riffles and shallow rapids upstream from camp, Waruma Creek	Guyana
ROMT12696	*T.* cf. *guianensis*	89932	4.95407	–59.85882	MT025539	MT017648	MT017620	MT017600	Potaro River drainage	At Kopinang Village landing, schoolhouse rapids, Kopinang River	Guyana

Table 1. Continued

Tissue catalogue	Species identity	Voucher number	Latitude	Longitude	16S	COI	Cytb	rag2	Drainage	Locality name	Country
ROMT15527	*T.* cf. *guianensis*	91392	5.272085861	−59.7026908	MT025540	MT017649	MT017621	MT017601	Potaro River drainage	At upstream-most rapid (Rapid 4), Upper Kuribrong River	Guyana
ROMT15575	*T.* cf. *guianensis*	91500	5.3759978	−59.5472803	MT025541	MT017650	MT017622	MT017602	Potaro River drainage	At top of Amaila Falls (Amaila River mouth), Amaila River	Guyana
ROMT15595	*T. conradi*	91436	5.413958782	−59.470252	MT025542	MT017651	MT017623	MT017605	Potaro River drainage	Approximatey 0.5 km upstream from mouth, at rapids beyond first rapid blocking upstream boat entry, Mikobe Creek	Guyana

those of Ochoa *et al.* (2017), alignments were trimmed to the following lengths: 16S, 466 bp; *COI*, 522 bp; *Cytb*, 858 bp; and *rag2*, 885 bp. Each individual gene tree was analysed with *Scleronema minutum* as an outgroup. The concatenated dataset (3579 bp) included members of clades D1, D2, D3 and E from the study by Ochoa *et al.* (2017), with *Scleronema minutum* as an outgroup. Data were exported both as individual gene alignments and as a concatenated dataset for phylogenetic analysis.

PHYLOGENETIC ANALYSIS

Best-fitting models of evolution were tested using PartitionFinder2 (Lanfear *et al.*, 2017). Models were tested on individual gene trees partitioned by codon, then on the concatenated dataset partitioned by gene. The resulting data blocks were then used in Bayesian inference analysis. Bayesian inference was performed using MrBayes v.3.2.6 on XSEDE via the CIPRES Science Gateway (Miller *et al.*, 2010). Each dataset had two runs with four chains run for 15 million generations, sampling once every 1000 generations. The parameters and trees were summed in MrBayes v.3.2.6 using the default 25% burn-in. The resulting 50% majority consensus rule phylogeny is reported. Convergence was also checked in TRACER v.1.7.1, with the requirement of an ESS value > 200. A maximum likelihood analysis was also performed on the concatenated dataset (Supporting Information, Fig. S1). Branch supports were obtained with the ultrafast bootstrap (Hoang *et al.*, 2018) implemented in the IQ-TREE software (Nguyen *et al.*, 2015).

RESULTS

The molecular analyses consistently recognized six species-level clades of *Trichomycterus* in the Pakaraimas: (1) *T. conradi*; (2) *T. guianensis*; (3) *T.* cf. *guianensis* (*sensu* Ochoa *et al.*, 2017); (4) Ireng spotted; (5) Potaro, elongate; and (6) Mazaruni, plain. Four gene trees were analysed separately, then combined into a concatenated analysis.

The first individual gene tree is cytochrome *b* (*Cytb*; Fig. 3A), which results in a well-supported clade (posterior probability > 90%) of Pakaraima *Trichomycterus*. Members of true *T. guianensis* are found sister to the Potaro, elongate form. This clade is sister to another well-supported clade [posterior probability (pp) > 90%] of *T.* cf. *guianensis* + Mazaruni, plain form. These are all sister to a single representative of *T. conradi*. This analysis did not include the Ireng, spotted form that is present in other analyses, owing to difficulties in amplifying and successfully sequencing them.
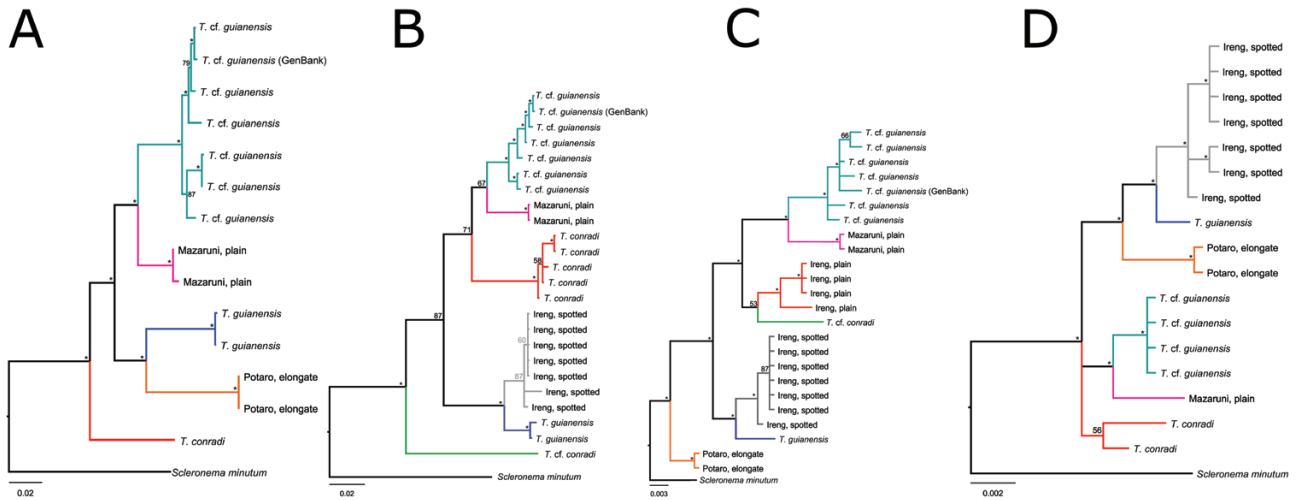
Figure 3. Fifty per cent majority rule consensus tree from Bayesian inference of each gene analysed in this study: cytochrome *b* (A), *COI* (B), 16S (C) and *rag2* (D). Nodes labelled with an asterisk indicate posterior probabilities > 90%. Values < 90% are written on the trees. Branches are coloured to match localities as seen in Figure 1. Tip labels correspond to individuals as denoted in Table 1.

The second gene tree generated from our data is based on *COI* (Fig. 3B). This analysis again places *T.* cf. *guianensis* sister to the Mazaruni, plain form. In contrast to the *Cytb* phylogeny, this clade is sister to *T. conradi*; however, this relationship is weakly supported (pp = 71%). The (*T.* cf. *guianensis* + Mazaruni, plain) *T. conradi* clade is sister to another clade consisting of the Ireng, spotted form and true *T. guianensis.* The interrelationships among the clades are poorly supported (pp = 67%), but each recognized morphotype is well supported (pp > 90%), with the exception of the Ireng, spotted form (pp = 87%). Finally, the Potaro, elongate form is missing from this analysis owing to difficulties in amplifying and successfully sequencing it. Overall, the *COI* tree is much less resolved than the other trees, with some nodes not reaching 90% posterior probability.

Ribosomal 16S data place *T.* cf. *guianensis* sister to the Mazaruni, plain form (Fig. 3C). This clade is sister to *T. conradi*. As seen in the *COI* analysis, despite geographical proximity, the Ireng, spotted form is sister to true *T. guianensis* rather than *T. conradi*. The Potaro, elongate form is sister to the remaining members of the Pakaraima member clade.

Nuclear DNA analysis from the *rag2* data was the most divergent from the remainder of the data (Fig. 3D). Again, *T.* cf. *guianensis* is recovered as sister to the Mazaruni, plain form, but this is the only similarity with the other gene trees. The *rag2* data show true *T. guianensis* as sister to the Ireng, spotted form. They form a clade sister to the Potaro, elongate form. The *T. conradi* is weakly monophyletic (pp = 56%), and its deeper relationships are unresolved owing to a polytomy.

With gene tree heterogeneity rampant in this analysis, the four genes were concatenated and analysed with the D1, D2, D3 and E clades from the study by Ochoa *et al.* (2017; Fig. 4). The Bayesian analysis resulted in two trees with equal likelihood. The two runs were examined separately, and a rogue taxon, *Ituglanis parahybae*, was the cause. Given that this taxon was not the focus of the present study and because the topology was otherwise unchanged, we present the 50% majority rule consensus tree. The maximum likelihood tree showed the same topology as the Bayesian 50% majority rule consensus tree and is included as a supplemental figure (Supporting Information, Fig. S1). The tree, with 24 individuals from our analysis, shows that all morphotypes we identified a priori are monophyletic. Two distinct clades compose Pakaraima *Trichomycterus*: *T.* cf. *guianensis* + Mazaruni, plain form is sister to *T. conradi*. This clade is sister to another clade consisting of *T. guianensis* + Ireng, spotted, which is sister to the Potaro, elongate form. Each of these relationships is supported with > 90% posterior probability, whereas deeper relationships remain unresolved.

DISCUSSION

Our results demonstrate the presence of multiple species of *Trichomycterus* in the Pakaraima Mountains of Guyana. Based on the concatenated analysis, there were two major clades, one consisting of *T. guianensis* and two undescribed species from the Potaro and Ireng Rivers (Ireng, spotted and Potaro, elongate), and the second clade including *T. conradi* and two undescribed
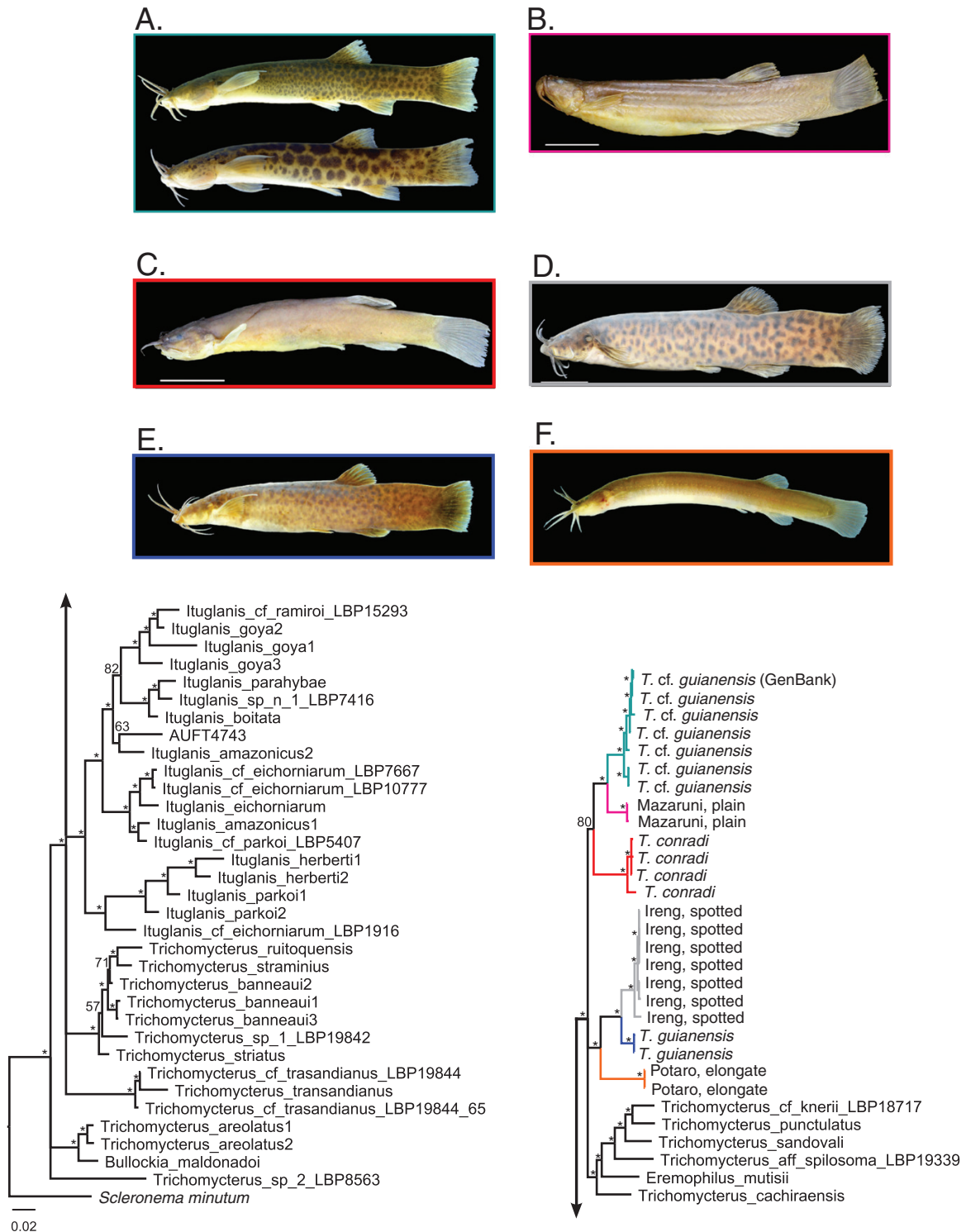
Figure 4. Fifty per cent majority rule consensus tree from Bayesian inference of concatenated sequences. Nodes labelled with an asterisk indicate posterior probabilities > 90%. Values < 90% are written on the trees. Branches are coloured to match localities as seen in Figure 1. Tip labels correspond to individuals as denoted in Table 1. These sequences are combined with the clades D1, D2, D3 and E from Ochoa *et al.* (2017). Arrows connect disconnected branches in the phylogeny. Outlines of the photographs of specimens correspond to clade colour and symbol colour in Figure 1. A, *Trichomycterus* cf. *guianensis*. B, Mazaruni, plain form. C, *Trichomycterus conradi*. D, Ireng, spotted form. E, true *Trichomycterus guianensis*. F, Potaro, elongate form.

species, one from Mazaruni River (Mazaruni, plain) and *T.* cf. *guianensis*. Both *T. guianensis* and *T. conradi* appear to be among the rarer species in the region (based on collections), and *T. guianensis* is only in the upper Potaro River. Based on preliminary examination of the types and comparison with specimens we have collected, *T. guianensis* is a deep-bodied species with irregular blotches (Fig. 4D). Ireng, spotted (Fig. 4E) is the dominant species in the Ireng, and it is similar in morphology to *T. guianensis* and was recovered as sister to it. In morphology, the Ireng spotted species is even shorter and deeper bodied than *T. guianensis*. Sister to the clade of *T. guianensis* and Ireng, spotted is a very elongate, almost entirely brown species from near Ayangana in the far upper Potaro with miniscule pelvic fins (= Potaro, elongate; Fig. 4F). It was found in sluggish, swampy areas, which is habitat more indicative of *Ituglanis*. *Ituglanis* was diagnosed (Costa & Bockmann, 1993) by the presence of a very small supraoccipital foramen (vs. large), an anteriorly directed anterior process of the sphenotic (vs. anteroventral) and a concave mesial margin of the autopalatine (vs. almost straight). Potaro, elongate has a very large foramen on the supraoccipital and an anteroventrally directed anterior process of the sphenotic, but it does share a concave mesial border of the autopalatine with *Ituglanis* (J.W.A., pers. obs.). In addition, *Ituglanis* has very few ribs, and a reduction in number to seven or fewer pairs was considered to be a synapomorphy for *Ituglanis* and a clade consisting of the subfamilies Tridentinae, Stegophilinae, Vandelliinae, Sarcoglanidinae and Glanapteryginae (Costa & Bockmann, 1993; De Pinna & Keith, 2003). Potaro, elongate has four pairs of ribs. Ochoa *et al.* (2017) found *Ituglanis* and *Trichomycterus* to be closely related and that *Ituglanis* is not sister to Tridentinae, Stegophilinae, Vandelliinae, Sarcoglanidinae and Glanapteryginae, suggesting that the number of ribs is homoplastic. Given the molecular phylogeny, the presence of synapomorphies of *Ituglanis* in Potaro, elongate would represent homoplasy, perhaps related to similar habitats, and we found Potaro, elongate to be related to other Pakaraima *Trichomycterus* in the molecular analysis. Potaro, elongate and Ireng, spotted are opposites in their morphology, with the former being deep bodied and short and the latter being long and slender, suggesting a strong capacity for body rearrangement in the genus. The morphological variation of all these species is currently under study by the authors, and descriptions and diagnoses are underway.

The other major clade contains a widespread, elongate species with dark, small or large, regular spots (*T.* cf. *guianensis*,; Fig. 4A). This species is found in the upper Potaro, Kuribrong and Mazaruni rivers. Little geographical structure was present in the specimens examined, suggesting fairly recent movement between the basins. Sister to this species is a similar but unspotted species from the Mazaruni River (Mazaruni, plain; Fig. 4B). Finally, a clade composed of the plain-coloured *T. conradi* (Fig. 4C) from the Ireng and the lower Kuribrong is sister to the other two species.

Two species of *Trichomycterus* have been described from the upper Caroní, Orinoco River basin section of the Pakaraima Mountains: *Trichomycterus celsae* Lasso & Provenzano, 2002 and *Trichomycterus lewi* Lasso & Provenzano, 2002. Based on preliminary examination of specimens at AUM, *T. celsae* is most similar to *T. conradi*, and *T. lewi* might be the same species as *T.* cf. *guianensis*. Further morphological investigation is underway to determine species identity. Unfortunately, we do not have tissue samples from the Venezuelan species.

BIOGEOGRAPHY OF THE PAKARAIMA MOUNTAINS

The biogeographical story that the species of *Trichomycterus* of the Pakaraimas tell is a complex one. *Trichomycterus* cf. *guianensis* appears to have moved between river systems with relative ease. Mazaruni samples are sister to those in the Kuribrong and Potaro rivers, but the Mazaruni samples are paraphyletic. Tributaries of the Mazaruni interdigitate with the Kuribrong and Potaro rivers, and species living as high in their drainages as *Trichomycterus* would be more likely to be able to move via river capture events, in which tributaries erode their divides and switch from one system to the next. Anecdotal reports suggest that the upper courses of at least the Potaro and Kuribrong connect during particularly rainy times; flying over the area reveals numerous fissures that seem to run between the two rivers (J.W.A., pers. obs.). These drainages also interdigitate with Caroní and Ireng tributaries. As mentioned above, *T.* cf. *guianensis* might be conspecific with *T. lewi* described from Venezuela; however, we found no similar species in the Ireng, suggesting a limit to the distribution of the species.

The upper Caroní and the Ireng were once part of the proto-Berbice palaeodrainage basin along with the upper Branco, upper Essequibo, Berbice and Courantyne rivers, while the Mazaruni was likely to be independent (Lujan & Armbruster, 2011). The Essequibo makes a westward bend near Massara and away from a nearby Berbice tributary (Gibbs & Barron, 1993), suggesting a likely point of demarcation between the upper Essequibo as part of the proto-Berbice and the lower Essequibo, which probably joined with the Mazaruni at the present mouth of the Essequibo. This would mean that the Potaro and Mazaruni were part of the same system and not part of the proto-Berbice. However, the mixing of Ireng, Potaro and Mazaruni

Trichomycterus in the phylogeny suggests that there probably existed faunal exchange between the proto-Berbice, Potaro and Mazaruni rivers, at least in the highlands before the break-up of the proto-Berbice during the Pliocene and Pleistocene, potentially leading to complex interrelationships between these basins. A similar finding was made by Lujan *et al.* (2019), who found that *Corymbophanes* was sister to a new genus and species from the Ireng (*Y. primus*); however, the branch lengths were much longer than what was observed here. Further exploration of the relationships of *Trichomycterus* along with a molecular clock will probably lead to fascinating insights into the biogeography of the Pakaraima Mountains, but this further insight will require extensive collecting in the Brazilian tributaries of the Pakaraima Mountains, which are difficult to explore, and further collecting in Venezuela, which is difficult now because of civil strife.

Although Ochoa *et al.* (2017) did perform a molecular dating analysis, only one of the two dates used for calibration was from a fossil, and that from only ~4.5 Mya. The other calibration point was based on an estimated age of the family from another study. The divergence date given by Ochoa *et al.* (2017) for *T.* cf. *guianensis* from a trans-Andean sister clade is 19.22 Mya. We felt as the only fossil was recent but from a distantly related clade, the ancient origin of the family was based not on fossils but on ages from another study, and there were likely to be large gaps in sampling between Pakaraima *Trichomycterus* and potentially related taxa, performing a molecular clock analysis here would be premature and potentially misleading.

Trichomycterus conradi appears to be a more lowland form, found in the rapids below Kaieteur and Amaila Falls on the Potaro and Kuribrong, respectively, and in the Ireng. The shallow nodes between the Kuribrong and Ireng samples sequenced suggest that movement has been relatively recent. We were able to obtain 16S sequences only for a specimen of *T.* cf. *conradi* from the Maroni River of eastern Suriname, and it was sister to *T. conradi*. A similar distribution across the northern Guiana Shield was found for *P. bovallii* from the Ireng River and hypothesized new species related to it in the Potaro, Courantyne and Coppename rivers (Lujan *et al.*, 2018). The distributions of *T. conradi* and *P. bovallii s.l.* suggest interconnectivity across the Guiana Shield even for small fishes restricted to fast-flowing streams. Clearly, we are only beginning to understand the complexities of the biogeography of the western Guiana Shield and the interconnectedness of it with the eastern portion of the shield.

THREATS TO BIODIVERSITY IN THE GUYANA HIGHLANDS

The Pakaraimas represent the cores of ancient mountains, which are among the main sources of gold and diamonds. Alofs *et al.* (2014) reviewed some of the issues with gold mining in the upper Mazaruni River, and we have observed similar issues in the Kuribrong and Potaro Rivers. Large swathes of forest have been removed from around the rivers, with the sediment pumped through sieves to extract gold and diamonds. Gold is removed with mercury amalgamation, leading to high mercury levels in the water, fishes and humans (Miller *et al.*, 2003) and large swathes of forest replaced by denuded landscapes and toxic spoil ponds. On larger rivers, such as the lower Potaro, large dredging machines suck up sediment and process it directly in the river, leaving behind piles of gravel in the river that alter the natural hydrology. Although Hardman *et al.* (2002) did not find significant differences between their study of the fishes of the Potaro River and the study by Eigenmann (1912), certain species that had been present and common in Eigenmann's survey were absent 90 years later. Mol & Ouboter (2004) and Brosse *et al.* (2011) found that the erosion related to gold mining has reduced fish diversity. As of our 2014 trip to the upper Kuribrong and 2016 trip to the Ireng, there was little impact to the rivers from mining; however, a recently completed road now provides easier access to the upper Kuribrong, and one small mine was observed in 2014. The lower Kuribrong has been heavily impacted, and after flying over the Potaro River in 2014, J.W.A. can state that the Potaro looks less clear than it did during the 1998 expedition reported by Hardman *et al.* (2002).

Although depauperate in total numbers of species, the high degree of endemism makes the Pakaraima Mountains a particularly interesting hotspot of biodiversity. As expressed by Alofs *et al.* (2014) for the upper Mazaruni, the whole high plateau of the Pakaraimas supports an endemic fauna, as is evidenced here. Although there is some interconnectivity of the river systems, narrow endemic *Trichomycterus* are found in each of the rivers in the present study. It is clear from the present study and those of López-Fernández *et al.* (2012) and Lujan *et al.* (2019) that the high Pakaraimas are acting as species generators, and the very factors that make the area difficult to colonize (such as large waterfalls) also lead to genetic isolation. In *Trichomycterus* alone, we know of the six species presented here, *T. celsae*, *T. lewi*, and at least one more undescribed species from Venezuela in the Pakaraimas. Few areas of this size outside of the Pakaraimas have such high diversity of *Trichomycterus*. Conservation of the unique landscape of the Pakaraimas that has become part of our shared cultural heritage is important, and further studies on the unique fauna of the region are needed.

ACKNOWLEDGEMENTS

REFERENCES

Alofs KM, **Liverpool EA**, **Taphorn DC**, **Bernard CR**, **López-Fernández H. 2014.** Mind the (information) gap: the importance of exploration and discovery for assessing conservation priorities for freshwater fish. *Diversity and Distributions* **20:** 107–113.

Armbruster JW, **Taphorn DC. 2011.** A new genus and species of weakly armored catfish from the Upper Mazaruni River, Guyana (Siluriformes: Loricariidae). *Copeia* **2011:** 46–52.

Baskin JN. 1973. *Structure and relationship of the Trichomycteridae*. New York: City University of New York, 389.

Brosse S, **Grenouillet G**, **Gevrey M**, **Khazraie K**, **Tudesque L. 2011.** Small-scale gold mining erodes fish assemblage structure in small neotropical streams. *Biodiversity and Conservation* **20:** 1013–1026.

Costa WJEM, **Bockmann FA. 1993.** Un nouveau genre Néotropical de la famille des Trichomycteridae (Siluriformes: Loricarioidei). *Revue Française d'Aquariologie* **20:** 43–46.

De Pinna M, **Keith P. 2003.** A new species of the catfish genus Ituglanis from French Guyana (Osteichthyes: Siluriformes: Trichomycteridae). *Proceedings of the Biological Society of Washington* **116**: 873–882.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32:** 1792–1797.

Eigenmann CH. 1912. *The freshwater fishes of British Guiana, including a study of the ecological grouping of species and the relation of the fauna of the plateau to that of the lowlands, Vol. 5*. Pittsburgh: Carnegie Institute.

Fricke R, **Eschmeyer WN**, **van der Laan R. 2018.** *Eschmeyer's catalog of fishes: genera, species, references*. Available at: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp

Gibbs A, **Barron C. 1993.** *The geology of the Guiana Shield*. Oxford: Oxford University Press.

Hardman M, **Page LM**, **Sabaj MH**, **Armbruster JW**, **Knouft JH. 2002.** A comparison of fish surveys made in 1908 and 1998 of the Potaro, Essequibo, Demerara, and coastal river drainages of Guyana. *Ichthyological Exploration of Freshwaters* **13:** 225–238.

Henschel E, **Mattos JLO**, **Katz AM**, **Costa WJEM. 2018.** Position of enigmatic miniature trichomycterid catfishes inferred from molecular data (Siluriformes). *Zoologica Scripta* **47:** 44–53.

Hoang DT, **Chernomor O**, **von Haeseler A**, **Minh BQ**, **Vinh LS. 2018.** UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* **35:** 518–522.

Irwin DM, **Kocher TD**, **Wilson AC. 1991.** Evolution of the cytochrome *b* gene of mammals. *Journal of Molecular Evolution* **32:** 128–144.

Katz AM, **Barbosa MA**, **de Oliveira Mattos JL**, **da Costa WJEM. 2018.** Multigene analysis of the catfish genus *Trichomycterus* and description of a new South American trichomycterine genus (Siluriformes, Trichomycteridae). *Zoosystematics and Evolution* **94:** 557–566.

Kearse M, **Moir R**, **Wilson A**, **Stones-Havas S**, **Cheung M**, **Sturrock S**, **Buxton S**, **Cooper A**, **Markowitz S**, **Duran C**, **Thierer T**, **Ashton B**, **Meintjes P**, **Drummond A. 2012.** Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28:** 1647–1649.

Kocher TD, **Thomas WK**, **Meyer A**, **Edwards SV**, **Pääbo S**, **Villablanca FX**, **Wilson AC. 1989.** Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of America* **86:** 6196–6200.

Lanfear R, **Frandsen PB**, **Wright AM**, **Senfeld T**, **Calcott B. 2017.** PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* **34:** 772–773.

López-Fernández H, **Taphorn DC**, **Liverpool EA. 2012.** Phylogenetic diagnosis and expanded description of the genus *Mazarunia* Kullander, 1990 (Teleostei: Cichlidae) from the upper Mazaruni River, Guyana, with description of two new species. *Neotropical Ichthyology* **10:** 465–486.

Lovejoy NR, **Collette BB. 2001.** Phylogenetic relationships of New World needlefishes (Teleostei: Belonidae) and the biogeography of transitions between marine and freshwater habitats. *Copeia* **2001:** 324–338.

Lujan NK, **Armbruster JW. 2011.** The Guiana Shield. In: Albert JA, Reis RE, eds. *Historical biogeography of Neotropical freshwater fishes*. Berkeley and Los Angeles, CA: University of California Press, 211–225.

Lujan NK, **Armbruster JW**, **Lovejoy NR. 2018.** Multilocus phylogeny, diagnosis and generic revision of the Guiana Shield endemic suckermouth armoured catfish tribe Lithoxini (Loricariidae: Hypostominae). *Zoological Journal of the Linnean Society* **184:** 1169–1186.

Lujan NK, **Armbruster JW**, **Werneke DC**, **Teixeira TF**, **Lovejoy NR. 2019.** Phylogeny and biogeography of the Brazilian–Guiana Shield endemic *Corymbophanes* clade of armoured catfishes (Loricariidae). *Zoological Journal of the Linnean Society* zlz090.

Lundberg JG, **Marshall LG**, **Guerrero J**, **Horton B**, **Malabarba MCSL**, **Wesselingh F. 1998.** The stage for neotropical fish diversification a history of tropical south american rivers. In: Malabarba LR, Reis RE, Vari RP, Lucena ZM, Lucena CAS, eds. *Phylogeny and classification of Neotropical fishes*. Porto Alegre, Brazil: Edipucrs, 13–48.

Miller JR, **Lechler PJ**, **Bridge G. 2003.** Mercury contamination of alluvial sediments within the Essequibo and Mazaruni River Basins, Guyana. *Water, Air, and Soil Pollution* **148:** 139–166.

Miller MA, **Pfeiffer W**, **Schwartz T. 2010.** Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*. New Orleans, LA, 1–8.

Mol JH, **Ouboter PE. 2004.** Downstream effects of erosion from small-scale gold mining on the instream habitat and fish community of a small Neotropical rainforest stream. *Conservation Biology* **18:** 201–214.

Nguyen LT, **Schmidt HA**, **Von Haeseler A**, **Minh BQ. 2015.** IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* **32:** 268–274.

Ochoa LE, **Roxo FF**, **DoNascimiento C**, **Sabaj MH**, **Datovo A**, **Alfaro M**, **Oliveira C. 2017.** Multilocus analysis of the catfish family Trichomycteridae (Teleostei: Ostariophysi: Siluriformes) supporting a monophyletic Trichomycterinae. *Molecular Phylogenetics and Evolution* **115:** 71–81.

Oliveira C, **Avelino GS**, **Abe KT**, **Mariguela TC**, **Benine RC**, **Ortí G**, **Vari RP**, **Corrêa e Castro RM. 2011.** Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingroup sampling. *BMC Evolutionary Biology* **11:** 275.

Palumbi SR, **Baker CS. 1994.** Contrasting population structure from nuclear intron sequences and mtDNA of humpback whales. *Molecular Biology and Evolution* **11:** 426–435.

de Pinna MCC. 2016. The dawn of phylogenetic research on Neotropical fishes: a commentary and introduction to Baskin (1973), with an overview of past progress on trichomycterid phylogenetics. *Neotropical Ichthyology* **14:** e150127.

Ribeiro AC. 2006. Tectonic history and the biogeography of the freshwater fishes from the coastal drainages of eastern Brazil: An example of faunal evolution associated with a divergent continental margin. *Neotropical Ichthyology* **4:** 225–246.

Schaefer CER, **do Vale JFJ. 1997.** Mudanças climáticas e evolução da paisagem em Roraima: uma resenha do Cretáceo ao Recente. In: Barbosa RI, Ferreira EJG, Castellón EG, eds. *Homem, Ambiente e Ecologia na Estado de Roraima*. Manaus: INPA, 231–265.

Sinha NKP. 1968. *Geomorphic evolution of the Northern Rupununi Basin, Guyana*. Savanna Research Series No 11. Montreal, Canada: Department of Geography, McGill University.

Taphorn DC, **Armbruster JW**, **López-Fernández H**, **Bernard CR. 2010.** Description of *Neblinichthys brevibracchium* and *N. echinasus* from the upper Mazaruni River, Guyana (Siluriformes: Loricariidae), and recognition of *N. roraima* and *N. yaravi* as distinct species. *Neotropical Ichthyology* **8:** 615–624.

Taphorn DC, **López-Fernández H**, **Bernard CR. 2008.** *Apareiodon agmatos*, a new species from the upper Mazaruni river, Guyana (Teleostei: Characiformes: Parodontidae). *Zootaxa* **1925:** 31–38.

Unmack PJ, **Bennin AP**, **Habit EM**, **Victoriano PF**, **Johnson JB. 2009.** Impact of ocean barriers, topography, and glaciation on the phylogeography of the catfish *Trichomycterus areolatus* (Teleostei: Trichomycteridae) in Chile. *Biological Journal of the Linnean Society* **97:** 876–892.

Ward RD, **Zemlak TS**, **Innes BH**, **Last PR**, **Hebert PDN. 2005.** DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences* **360:** 1847–1857.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Maximum Likelihood tree inference of concatenated sequences. Nodes labeled with an asterisk (*) indicate bootstrap support values >90%. Values less than 90% are written on the trees. Branches are colored to match localities as seen in Figure 1. Tip labels correspond to individuals as denoted in Table 1. These sequences are combined with the D1, D2, D3, and E clades from Ochoa *et al*. (2017).

SHARED DATA

Sequence data are available in GenBank. Accession numbers for each specimen and gene are listed in Table 1.